Inhalable powder formulation of a stabilized vasoactive intestinal peptide (VIP) derivative: Anti-inflammatory effect in experimental asthmatic rats

Shingen Misaka a, Yosuke Aoki a, Shin-ichiro Karaki b, Atsukazu Kuwahara b, Takahiro Mizumoto c, Satomi Onoue a,*, Shizuo Yamada a

a Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
b Laboratory of Physiology, Graduate School of Nutritional and Environmental Sciences, Institute for Environmental Sciences, University of Shizuoka, 52-1, Yada, Suruga-ku, Shizuoka 422-8526, Japan

c Product Development Section, ILS Inc., 1-2-1, Kubogooka, Moriya, Ibaraki 302-0104, Japan

ABSTRACT

Vasoactive intestinal peptide (VIP) exerts immunomodulating and anti-inflammatory activities through its specific receptors, such as VPAC1 and 2 receptors. Previously, a stabilized VIP derivative, [R15,20,21, L17]-VIP-GRR (IK312532), was proposed as a candidate of anti-asthma drug, and a dry powder inhaler system of IK312532 was also developed for inhalation therapy with minimal systemic side-effects. In the present study, the anti-inflammatory properties of IK312532 respirable powder (RP) were characterized in an asthma/COPD-like animal model, with the use of newly developed ovalbumin (OVA)-RP for lung inflammation. Marked inflammatory events in the lung were observed after OVA-RP challenge in rats as evidenced by significant increase of inflammatory biomarkers such as eosinophil peroxidase (EPO), myeloperoxidase (MPO) and lactate dehydrogenase (LDH). However, intratracheal administration of IK312532-RP led to significant attenuation of plasma EPO, MPO and LDH activities, as well as significant reduction of recruited inflammatory cells in BALF, especially macrophages and eosinophils. In the rats pretreated with IK312532-RP, histological examinations revealed that the inflammatory cells infiltrating to the lung and the epithelial wall thickness decreased significantly by 85% and 58%, respectively. Thus, inhalable powder formulation of IK312532 exerts its anti-inflammatory activity by suppressing granulocyte recruitment to the lung and epithelial hyperplasia, followed by the reduction of cytotoxic peroxidases.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Vasoactive intestinal peptide (VIP), an octacosapeptide, is a member of the glucagon–secretin superfamily [28]. VIP is found to have broad distribution in the body, such as the heart, lung, digestive and genitourinary tract, eyes, skin, ovaries and thyroid gland [34], and it has pleiotropic physiological effects, being involved in metabolic processes, exocrine and endocrine secretions, smooth muscle relaxation, neuroprotection, cell differentiation and the regulation of immune response [25]. In particular, VIP has been identified as a potential drug candidate for several chronic inflammatory diseases such as rheumatoid arthritis [4], asthma [27] and COPD [12,24], because of its potent anti-inflammatory efficacy. However, VIP is susceptible to proteolytic degradation in vivo after systemic administration, resulting in low potency and short duration of action in clinical application [10]. There is also a possibility that systemic dosing of VIP may cause moderate hypotension and/or other adverse effects. To overcome these drawbacks, metabolically stable derivatives of VIP and an effective drug delivery system for VIP need to be developed [23].

A number of stabilized VIP derivatives have been proposed so far, and their pharmacological properties were well characterized for the treatment of type 2 diabetes or asthma [13,17,33]. Previously, our group developed a VIP derivative, [Arg 15,20,21, Leu 17]-VIP-GRR (IK312532), with the aim of enhanced stability against the enzymatic digestion and prolonged duration of action [14]. IK312532 was found to be a potent nonselective VIP receptor agonist, and exerted smooth muscle relaxation in guinea-pig isolated trachea [18] and protective effects against the cytotoxicity of cigarette smoke extract in the rat alveolar L2 cells [20]. In addition, a novel dry powder inhaler (DPI) system for IK312532 was developed for pulmonary delivery [19], and intratracheal administration of IK312532 respirable powder (RP) resulted in marked attenuation of antigen-evoked infiltration of granulocytes...
in rat bronchial mucosa [18]. The mucosal inflammation is a characteristic feature of bronchial asthma and COPD, predominantly with T lymphocyte activation, tissue eosinophilia for asthma and neutrophilia for COPD [2]. Thus, the combined use of a long-acting VIP analogue and a pulmonary delivery system might be advantageous for the therapy of pulmonary inflammation diseases. However, the anti-inflammatory properties of IK312532, such as mechanism, types of targeted immune cells and duration of action have not been fully clarified.

The present study was conducted for further characterization of the anti-inflammatory properties of IK312532-RP in asthma/COPD model rats, prepared with inhalable ovalbumin (OVA) powder, as assessed by biomarker profiling and histochemical experiments. Plasma inflammatory biomarkers such as myeloperoxidase (MPO), eosinophil peroxidase (EPO) activities and lactate dehydrogenase (LDH) leakage were monitored after intratracheal administration of IK312532-RP in experimental asthma/COPD model rats. Histochemical evaluation was also carried out to assess the morphological changes and inflammatory events in the pulmonary tissues.

2. Materials and methods

2.1. Materials

OVA and aluminum hydroxide (alum) gel were purchased from Sigma Aldrich (St. Louis, MO). LDH from chicken heart, o-phenylenediamine (OPD), 10% formalin neutral buffer solution and trypan blue were bought from Wako Pure Chemical Industries (Tokyo, Japan). 3,3',5,5'-Tetramethylbenzidine (TMBZ) was obtained from Dojindo Laboratories (Kumamoto, Japan). 3,3'-Diaminobenzidine (DAB) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Erythritol was supplied by Nikken Chemicals (Tokyo, Japan) and all other reagents were obtained from commercial sources.

2.2. Preparation of dry powder formulations

Dry powder formulation of OVA and IK312532 was prepared as described previously [7,19]. Briefly, OVA or IK312532 was firstly ground with excipient to fine powders using a pestle and mortar and then milled with use of an A-O JET MILL (Seishin Enterprise, Tokyo, Japan) at a pusher nozzle pressure and grinding nozzle pressure of 0.60 and 0.55 MPa, respectively. The ratio of the compounds to excipient was 1:400 (w/w). The micronized materials were decompounded with 10-fold carrier particles (erythritol) in a plastic bag for 3 min, and the obtained dry powders of OVA and IK312532 were stored in a vacuum desiccator until testing.

2.3. Experimental procedure

Male Sprague–Dawley rats, weighing ca. 400 g (11 weeks of age) (Japan SLC, Shizuoka, Japan), were maintained at 24 ± 1°C under a cycle of 12 h light: 12 h darkness. Foods and water were supplied until testing. Male Sprague–Dawley rats, weighing ca. 400 g (11 weeks of age) (Japan SLC, Shizuoka, Japan), were maintained at 24°C under a cycle of 12 h light: 12 h darkness. Foods and water were supplied until testing. Male Sprague–Dawley rats, weighing ca. 400 g (11 weeks of age) (Japan SLC, Shizuoka, Japan), were maintained at 24°C under a cycle of 12 h light: 12 h darkness. Foods and water were supplied until testing. Male Sprague–Dawley rats, weighing ca. 400 g (11 weeks of age) (Japan SLC, Shizuoka, Japan), were maintained at 24°C under a cycle of 12 h light: 12 h darkness. Foods and water were supplied until testing.

2.4. Preparation of lung homogenate

Right middle lobe (ca. 0.2 g) of lung was removed after BAL, and then the lung was homogenized in 1 mL of 20 mM phosphate buffer (pH 7.4) and centrifuged (10,000 × g) for 10 min at 4°C. The resulting pellet was resuspended in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecylmethylammonium bromide. The suspension was subjected to four cycles of freezing and thawing, and disrupted by sonication for 40 s. After centrifugation (10,000 × g) for 5 min at 4°C, the supernatant was used for biochemical assays.

2.5. Histochemical analysis of lung tissue

Left lung lobes were removed after BAL and fixed in 10% formalin neutral buffer solution. Fixed tissues were washed with PBS (3 × 10 min) and soaked in PBS containing 30% sucrose and 0.1% sodium azide at 4°C for 24 h. The tissues were then embedded in O.C.T. Compound (Sakura Finetek, Tokyo, Japan), frozen in liquid nitrogen, and cut into the 12-μm-thick sections in a cryostat. For peroxidase–hematoxylin staining, sections on glass slides were dried, washed with diluted water and then reacted for 3 min in a solution of 0.05 M Tris–HCl buffer (pH 7.6) containing 14 mM DAB and 4 × 10−3% H2O2. Then, the sections were stained with hematoxylin for 1 min. For hematoxylin–eosin (HE) staining, sections were stained hematoxylin and eosin for general morphological changes in bronchial epithelium as assessed by epithelial wall thickness. Finally, sections were dehydrated and mounted using Mount Quick™ (Daido Sangyo, Saitama, Japan).

2.6. Measurement of MPO, EPO and LDH activities

Enzymatic detection of MPO activity in plasma and BALF was performed according to the previous report [3] with minor modification. In brief, assay mixtures consisted of 40 μL of H2O2 (final concentration 0.3 mM) in 80 mM sodium phosphate buffer (pH 5.4) and 50 μL plasma or lung homogenate samples. The reaction was initiated by addition of 10 μL TMBZ (final concentration 1.6 mM) in dimethyl sulfoxide at 37°C, and stopped after 2 min by the addition of 0.18 M H2SO4. Subsequently, optical density was determined at 450 nm. Detection of EPO activity in plasma and BALF was carried out as described previously with minor modification [35]. The reaction mixture was prepared by adding 500 μL OPD (50 mM) to 24.25 mL Tris buffer (pH 8.0), 3 μL 30% H2O2 and 25 μL Triton X-100. Then, 100 μL of reaction mixture was added to 50 μL of biological fluid sample in a 96-well plate and incubated for 30 min at room temperature. The reaction was stopped by the addition of 2 M H2SO4, and an absorbance at 490 nm was measured. A titration curve of horseradish peroxidase was used for the calculation of MPO and EPO activities, which were expressed in arbitrary units. LDH leakage to plasma was determined using the Wako LDH-Cytotoxic test (Wako Pure Chemical, Osaka, Japan), according to the manufacturer’s directions. LDH from chicken heart was used as the standard. Protein concentration in biological fluids was determined according to Lowry's method using a DC protein assay kit (Bio-Rad Laboratories, Hercules, CA) in accordance with manufacture instructions. All
samples were assayed in duplicate, and optical densities in all assays were measured by a microplate reader, Safire (Tecan, Männedorf, Switzerland).

2.7. Statistical analysis

The data were presented as mean ± SEM. One-way analysis of variance test (ANOVA) followed by Tukey’s test was used for statistical analysis. A P value of less than 0.05 was considered significant for all analyses.

3. Results

3.1. Histochemical changes in airway systems

OVA, a major egg white protein, has been used for preparation of experimental inflammatory animals [18,29,35]. Previously, a dry powder inhaler system of OVA exhibited suitable physicochemical properties for an inhalation system with high stability and dispersibility [16]. In this study, morphological changes in respiratory systems were evaluated firstly to ascertain the anti-inflammatory effects of IK312532-RP. The left lobe of the lung was sectioned at 24 h after OVA-RP challenge, and subjected to HE staining for the detection of granulocyte infiltration in airway systems. According to images of pulmonary tissues stained with HE, numerous cells were infiltrated into the bronchiolar sub-mucosa in rats treated with OVA-RP, as compared to rats treated with control-RP (Fig. 1). Conversely, in the IK312532-RP-pretreated group, the cellular infiltration was significantly attenuated by 85%, as compared with that in OVA-RP group (Table 1). In addition, the mean thickness of the epithelial wall was 2.4-fold increased after OVA-RP challenge in comparison with control-RP group, suggesting epithelial hyperplasia. However, pretreatment with IK312532-RP resulted in 58% reduction of the epithelial wall thickness. Furthermore, peroxidase staining of lung tissue revealed that the infiltrated cells mainly constituted of granulocytes, including eosinophils and neutrophils in the bronchial epithelium and interstitium as shown in Fig. 2. This infiltration of granulocytes was markedly (98%) decreased by inhaled IK312532-RP, suggesting that IK312532-RP strongly inhibited the recruitment of the immune cells to the bronchus and bronchiolo.

3.2. Recruitment of granulocytes in BALF

BALF has been frequently used as a biological source for diagnosis or investigation of inflammatory lung diseases [30,32]. In the present study, BALF was obtained at 24 h after OVA-RP challenge, and the cells contained in BALF were counted after Wright–Giemsa staining to identify the type of inflammatory cells (Fig. 3). OVA-RP challenge in antigen sensitized rats led to a significant increase of cell numbers as compared to control group (OVA group, 80.2 ± 17.4 × 10^5 cells/mL; and control group, 24.0 ± 4.6 × 10^5 cells/mL). However, no increase of cell numbers was observed in the IK312532-RP pretreated group (19.5 ± 7.6 × 10^5 cells/mL) (Fig. 3). The numbers of macrophages or eosinophils in BALF, retrieved from OVA-RP challenged rats, were higher than those in control rats by 3.2- and 6.3-fold, respectively (Table 2). On the other hand, pretreatment with IK312532-RP led to marked reductions in the numbers of macrophages and eosinophil, but not neutrophils. These findings indicated that IK312532 might have an immunomodulating effect.

3.3. Biomarker profiling in OVA-RP induced lung inflammation

3.3.1. MPO activity

MPO is one of the pro-inflammatory and pro-oxidant enzymes, mainly released from activated neutrophils and macrophages [36]. Previously, our group demonstrated that MPO activity in plasma was markedly increased after OVA-RP challenge, suggesting that it could be a sensitive biomarker associated with OVA induced lung inflammation [16]. As shown in Fig. 4, the plasma MPO level was immediately increased after OVA-RP challenge and reached a maximum level at 12 h. In contrast, MPO activity in rats pretreated with IK312532-RP before OVA-RP challenge was significantly reduced to baseline level during the experimental period. MPO activity in BALF also resulted in a 94% decrease by IK312532-RP pretreatment compared with the OVA-RP alone group (Table 2).

3.3.2. EPO activity

EPO is produced and released by eosinophils and acts as a pro-inflammatory mediator [8]. EPO activity has thus been suggested as an inflammation biomarker of eosinophil activation, possibly reflecting the degree or type of the inflammation [35]. In the present investigation, LDH release was monitored after OVA-RP challenge. Similar to the previous reports, treatment with OVA-RP led to a biphasic increase of LDH activity, but not neutrophils. These findings indicated that IK312532 might have an immunomodulating effect.

3.3.3. LDH leakage

LDH is a cytoplasmatic enzyme present in essentially all major organ systems and the extracellular appearance of LDH has been used to detect cell damage or cell death [9]. Thus, the measurement of LDH is considered to be a sensitive indicator of lung damage or inflammation [6]. In the present investigation, LDH release was monitored after OVA-RP challenge. Similar to the previous reports, treatment with OVA-RP led to a biphasic increase of LDH activity.

Fig. 1. Morphological changes of bronchial epithelium in rats after inhalation of OVA-RP with or without IK312532-RP administration. Lung tissues were fixed with 10% buffered formalin, sectioned to a thickness of 12 μm, and stained with hematoxylin–eosin. (A) Control, (B) rats treated with OVA-RP and (C) rats treated with IK312532-RP and OVA-RP. Each scale bar represents 100 μm.
reaching maximum levels at 3 and 24 h in plasma (343% and 371% increment, respectively) (Fig. 6). The elevated LDH activities in the early and late phase were reduced to the control levels by pretreatment with IK312532-RP.

4. Discussion

In the present study, we demonstrated the anti-inflammatory properties of IK312532, a stabilized VIP derivative, on OVA-RP-induced lung inflammation in rats. Pretreatment with inhaled IK312532-RP in OVA-sensitized rats significantly attenuated antigen-induced increase of infiltrated granulocytes in the bronchial submucosa, epithelial wall thickness, total cell number in BALF, and the inflammation-related biomarkers in plasma. These observations suggest the mechanism and therapeutic potential of specific VIP receptor agonists for the treatment of inflammatory lung diseases such as asthma and COPD.

According to the structure-activity relationship studies of VIP, IK312532 was developed as nonselective agonist for VIP receptors with high affinity to its receptors and enhanced stability in the biological environment [18]. The pharmacological effects of VIP are mediated through three G protein-coupled receptors, including PAC1, VPAC1 and VPAC2, and these receptors are distributed very broadly in the body [26,31]. In particular, VPAC1 receptor is constitutively expressed in both unstimulated and stimulated T cells and macrophages, while the predominant expression of VPAC2 receptor is confirmed in the smooth muscle layers and the basal part of the mucosal epithelium in the lung, but not in unstimulated T cells [5]. In the immune system, VIP is currently identified as an immunomodulatory neuropeptide, because of its multiple anti-inflammatory effects [11]. Previously, its therapeutic potential has been reported in several murine models of inflammatory and autoimmune disease, including septic shock, rheumatoid arthritis and Crohn's disease [1,4,15]. In these models, treatment with VIP led to marked decreases in the levels of pro-inflammatory mediators by modulating the Th1/Th2 balance. The important roles of VIP as an endogenous anti-inflammatory agent were also supported by pathophysiological features of VIP gene knockout mice, in which severe inflammation occurs in the lung [32]. Therefore, VIP and its derivatives have been proposed as promising candidates for the treatment of inflammatory lung diseases [12,25], and several VIP derivatives are currently under clinical and pre-clinical development [1,13,30,33]. Previously, our group also proposed the new DPI system of IK312532 for topical administration to the lung with the use of jet-milling technology [19]. Unlike oral and injectable formulations, the DPI formulation has several advantages, including the avoidance of aggregation in liquid state, the evasion of first pass hepatic metabolism and maximal local effect in the target organ with minimal systemic side-effects [21]. The inhalable formulation of IK312532 exhibited high emission from the capsule and deposition in the airway systems, and the aerodynamic diameter of jet-milled particles was

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Morphological changes in the lung after OVA-RP challenge.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>OVA-RP</td>
</tr>
<tr>
<td>Number of infiltrated cells ($\times 10^6$ cells/mm$^2$)</td>
<td>29.3 ± 2.9</td>
</tr>
<tr>
<td>Wall thickness (μm)</td>
<td>21.3 ± 2.3</td>
</tr>
</tbody>
</table>

Lung tissues of rats, pretreated with IK312532-RP, followed by OVA-RP administration, were fixed with 10% neutral buffered formalin, sectioned to a thickness of 12 μm, and stained with hematoxylin–eosin (HE) staining. Data represent mean ± SEM (n = 3–6).

* p < 0.05 with respect to control-RP.
# p < 0.05 with respect to rats treated with OVA-RP.

Fig. 2. Determination of infiltrated granulocytes in the lung after inhalation of OVA-RP with or without IK312532-RP administration. Lung tissues were fixed with 10% buffered formalin, sectioned to a thickness of 12 μm, and stained with DAB and hematoxylin. (A) Control, (B) rats treated with OVA-RP, (C) rats treated with IK312532-RP and OVA-RP and (D) the numbers of recruited granulocytes. Each scale bar represents 100 μm. Data represent the mean ± SEM of 5–6 determinations. ** p < 0.01 with respect to vehicle + OVA-RP challenged rats.
found to range from 1 to 5 μm \[19\]. These physicochemical properties suggested that the IK312532-DPI system could be suitable for pulmonary delivery with the aim of avoiding systemic side-effects.

In our previous study, IK312532 potently attenuated the cytotoxicity induced by exposure to cigarette smoke extracts through the deactivation of caspase-3 in rat alveolar L2 cells in vitro \[20\]. Intratracheal administration of IK312532-RP markedly suppressed the antigen-evoked infiltration of granulocytes in the rat bronchiolar mucosa as well as intraperitoneal administration of dexamethasone \[18\]. The present study demonstrated that pretreatment with IK312532 before antigen challenge resulted in significant reductions of inflammatory biomarkers such as EPO, MPO and LDH in plasma, in agreement with the remarkable inhibition of the recruitment of immune cells in the lung and BALF. In the BALF samples, the inhaled IK312532-RP reduced the elevated levels of EPO and MPO activities by 83 and 94%, respectively, as compared to the OVA-sensitized group. These observations were partly consistent with previous observations that a synthetic VIP analogue inhibited IL-1β-induced neutrophil recruitment in the airway \[30\]. In addition to inflammatory events, the extracellular LDH was determined in the present study to assess cell damage or cell death in the pulmonary tissues \[6\]. Although biphasic elevation of LDH was observed after OVA-RP challenge, the pretreatment with IK312532-RP led to a marked reduction of LDH in both early and late phases. The result supported the inhibitory effect of IK312532-RP on inflammatory cell recruitment, leading to protection of airway tissues from oxidative stresses and other toxic responses. On the basis of these data, taken together with the well-established pathogenetic process of airway inflammation \[22\], the anti-inflammatory action of IK312532 might be directly associated with modulation of cell chemotaxis and/or inflammatory chemokines from activated cells, which might lie in the upstream of airway inflammatory cascade.

Asthma and COPD are both characterized by chronic inflammation of the respiratory tract, mediated by the increased expression of multiple inflammatory molecules \[2\]. Despite the similarity of some clinical features in these two diseases, there are marked differences in their pathological features. In asthmatic patients, there is an increase in the number of Th2 cells, a central role of which is regulation of allergic inflammation by secreting several cytokines such as IL-4 and IL-5. It was reported that the IL-5...
Data represent the mean treated with OVA-RP.

In contrast, macrophages and Th1 cells are responsible for inflammatory responses in patients with COPD, and they facilitate the differentiation of neutrophils via releasing several chemokines. According to the results from BALF analysis (Table 2), OVA-RP challenge caused eosinophil-predominant inflammatory events, reflecting the clinical condition of asthma. Marked elevation of plasma MPO was seen after OVA-RP challenge, whereas there was only a slight increase in neutrophils and MPO levels in BALF, the typical biomarkers for COPD. Interestingly, IK312532-RP could suppress the symptoms of inflammation as evidenced by the significant reduction of eosinophils in BALF and EPO activity in plasma. Based on these findings, inhalation therapy using IK312532-RP might be effective for the treatment of asthma.

However, the therapeutic potential for COPD is still unclear because of the limited experimental model. Further investigation will be required to validate the therapeutic potential of IK312532 on COPD, with the use of well-designed experimental animal models such as the chronic tobacco smoke exposure model.

In conclusion, IK312532-RP could ameliorate OVA-RP-induced inflammatory responses in the lung, supported by morphological and histochemical alteration, cellular counting in BALF, and biomarker profiling in rats. These findings suggest a possible mechanism for the anti-inflammatory effect of IK312532, which includes the modulation of cellular chemotaxis or much earlier signaling cascade of inflammation. IK312532 could be a therapeutic drug candidate for the treatment of asthma and COPD, particularly in combination with use of a dry powder inhaler system.

Acknowledgement
This work was supported in part by a Grant-in-Aid for Young Scientists (B) (No. 20790103; S. Onoue) from the Ministry of Education, Culture, Sports, Science and Technology.

References


Table 2
Inflammatory responses in BALF.

<table>
<thead>
<tr>
<th>Recruited cells ($\times 10^6$)</th>
<th>Inflammatory biomarkers (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macrophages</td>
</tr>
<tr>
<td>Control</td>
<td>0.59 ± 0.32</td>
</tr>
<tr>
<td>OVA-RP with Vehicle</td>
<td>1.91 ± 0.76</td>
</tr>
<tr>
<td>IK312532-RP</td>
<td>1.18 ± 0.28</td>
</tr>
</tbody>
</table>

Each BALF was stained by Wright–Giemsa method to classify the type of inflammatory cells. A titration curve of horseradish peroxidase was used for the calculation of eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activities in BALF, expressed as arbitrary units. Data represent mean ± SEM (n = 3–6).

* p < 0.05 with respect to control.

# p < 0.05 with respect to rats treated with OVA-RP.

Fig. 5. Eosinophil peroxidase (EPO) activity in rat plasma after intratracheal administration of OVA-RP with or without IK312532-RP pretreatment. (△) Control, (●) rats treated with OVA-RP and (▼) rats treated with IK312532-RP and OVA-RP. Data represent the mean ± SEM of 5–6 determinations. * p < 0.05 with respect to rats treated with OVA-RP.

Fig. 6. Lactate dehydrogenase (LDH) activity in rat plasma after intratracheal administration of OVA-RP with or without IK312532-RP pretreatment. (△) Control, (●) rats treated with OVA-RP and (▼) rats treated with IK312532-RP and OVA-RP. Data represent the mean ± SEM of 5–6 determinations. * p < 0.05 with respect to rats treated with OVA-RP.


学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具